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Isolation and Preliminary Characterization of *Staphylococcus aureus* Lytic Phages from Wastewater Environment in Bobo-Dioulasso, Burkina Faso, West Africa

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Abstract

Staphylococcus aureus (*S. aureus*) is a bacterial pathogen for humans and animals. These bacteria can resist against many antibiotics and this resistance constitute an alarming worldwide human health threat due to the morbidity and mortality. Phage therapy is one of the alternative treatments. The aim of this study was to isolate and characterize lytic phages of *S. aureus* from different wastewater sources in Bobo-Dioulasso, Burkina Faso. Eight strains of *S. aureus* were isolated from different clinical samples and were used to isolate phages. The isolation and host range of phages were done by the spot test. Phages were purified by the double-layer method. Similar phages after the determination of the host range were characterized using restriction enzymes. A total of 27 phages were obtained after isolation and purification. Nine of the 27 isolates reported a broad host range (≥67%). The results of enzymatic digestion allowed to consider that all phage isolates that presented the same host range and the same genetic fingerprint are the same phage strain; whereas phages that presented the same host range and different genetic fingerprints are different phage strains. Thus, a total of 15 distinct phages isolates specific to *S. aureus* were characterized. This study highlighted the abundance and lytic capacity of phages isolated from wastewater from Bobo-Dioulasso's environment against clinical strains of *S. aureus*. The lytic capacity of these

Staphyphages could be an effective alternative tool to combat bacteria multi-resistance.

Keywords

Staphylococcus aureus, Lytic Phages, Wastewater, Isolation, Burkina Faso

1. Introduction

Staphylococcus aureus (*S. aureus*) are Gram-positive cocci that are both commensal and pathogenic in humans and animals. They are one of the main bacteria responsible for respiratory infections, endocarditis, bacteremia, as well as osteoarticular, skin and medical device infections [1] [2].

As with several pathogenic bacterial species in recent years, the treatment of *S. aureus* infections has been characterised by the development and spread of resistance to many classes of antibiotics. Antimicrobial resistance (AMR) to drugs is now a global public health threat that requires urgent solutions, such as the development of new drugs or alternative solutions to antimicrobials. The spread of this AMR constitute a critical and persistent challenge to global health and modern health care [3].

Several studies have reported the burden of AMR at global, regional and national levels with different situations across continents and countries.

A global assessment of the burden of AMR has estimated 4.95 million deaths associated with bacterial AMR and 1.27 million deaths attributable to bacterial AMR in 2019, with a disproportionate burden in low- and middle-income countries. For example, 27.3 deaths per 100,000 in western Sub-Saharan Africa and 6.5 deaths per 100,000 in Australasia [3]. A more precise estimate at regional level shows that 1.05 million deaths were associated with bacterial AMR, and 250,000 deaths were attributable to bacterial AMR in the WHO African Region in 2019 [3].

If nothing is done to reverse the situation, it is estimated that AMR will increase steadily over time, resulting in more than 10 million deaths per year by 2050 [4]. Resistance to antibiotics is found in most pathogenic bacteria, but at different levels depending on the bacterium and the drug. In 2019, there were 33 bacterial pathogens, including pathogenic resistant bacteria that caused the majority (7.7 of 13.7 million) of deaths related to infectious diseases [5]. Among these 33 bacterial pathogens, six leading pathogens *i.e.*, *Escherichia coli*, *S. aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* were responsible for more than 900,000 deaths attributable to AMR and 3.57 million deaths associated with AMR in 2019 [3].

For *S. aureus* (SA), its resistance concerns each class of antistaphylococcal drugs including penicillins, sulfonamides, tetracyclines, glycopeptides and others, complicating treatments. These strains identified in the 1960s were often resistant to

methicillin, an antibiotic with a β-lactam nucleus active against staphylococci [1]. They were first hosted in patients' homes and then confined in hospitals. In 1990, they appeared in the community under the name of Methicillin-resistant *S. aureus* (MRSA). MRSA is an MDR organism of great concern in the clinical setting as it is responsible of invasive infections and is the primary cause of hospital acquired infections [6].

MRSA developed resistance mechanisms to β-lactams with associated resistance to other families of antibiotics. Which complicates the clinician's therapeutic arsenal and represents a heavy burden for the patient and the health authorities [7] [8].

Burkina Faso, like many other countries, is facing a steady increase in multidrug-resistant bacteria from several species of bacterial pathogens. Recent reports of antimicrobial resistance surveillance in laboratory from the Ministry of Health and Hygiene of Burkina Faso highlight this alarming situation of AMR [9] [10]. For *S. aureus*, the 2024 report, concerning data of 2023 (data from 22 sentinel site laboratories across all Burkina Faso) showed resistance to many antibiotics. Out of the 1169 clinical isolates of *S. aureus* as part of this surveillance of antibiotic resistance in 2023, around 96%, 42%, 31%, 30%, 29%, 28%, 20%, 18%, 17% and 9% were resistant to respectively penicillin G, erythromycin, kanamycin, cefoxitin, ciprofloxacin, sulfamethoxazole + trimethoprim, clindamycin, gentamycin, tobramycin, and fusidic acid. Only the linezolid antibiotic was effective at 100% on all isolates (Ministry of Health and Public Hygiene, 2024). In addition, the frequency of global MRSA in Burkina Faso was 21.8% [10] and the frequency of MRSA bacteremia in patients was 69% [11].

The risk of death in people infected with methicillin-resistant *S. aureus* (MRSA) is 64% higher than in those infection reacts to drugs [12].

All these data on antimicrobial resistance demonstrate the existence of an extremely significant threat to global public health, which requires effective solutions in order to avoid health disasters in the future.

A number of strategies have been implemented, including research into new medicines by pharmaceutical companies and public research, regulations on the proper use of medicines, prevention of infections, vaccine development, Phage-based therapies [3] [13].

Several of these strategies are struggling to deliver the expected results, and AMR is only expanding in time and space. For example, the discovery of new antibiotics active against multiresistant bacteria is a major challenge due to the difficulties linked to the design of products with adequate physicochemical properties and acceptable toxicity profiles [14]. In addition, low and middle-income countries depend heavily on industrialized countries for their supply of medicines, with enormous difficulties and when global epidemics occur, developing countries receive essential medicines much later than industrialized countries [13]. Importantly for developing countries to avoid the catastrophe with AMR in the future they must gain greater autonomy and capacity to product and manufacture

antibiotic alternatives for their own populations [13].

However, the use of phages to control multidrug-resistant bacteria is a very promising approach, and one that is well suited to low and middle-income countries [13].

Phages or bacteriophages are virus that can specifically infect and kill susceptible and resistant bacteria and phage-based therapy were previously used before antibiotics era since their discovery in 1915 by Frederick Twort and named bacteriophages by Felix d'Herelle in 1917. But, they were rapidly abandoned due some difficulties, the discovery and mass production of effective antibiotics [15]. Bacteriophage are ubiquitous viruses, abundant in wastewater that specifically infect and lyse host bacteria [16] [17].

To date, a great deal of research on phages has been carried out in developed countries with the creation of phage banks, but rarely in Africa [13].

The aim of our study is to isolate and characterize from wastewater lytic bacteriophages of MRSA/SA, a highly worldwide concern pathogen. The ultimate goal is to build up the capacity for a bank of broad-spectrum lytic bacteriophages of priority pathogens.

2. Materials and Methods

2.1. Bacterial Strains

We used *S. aureus* strains isolated from various clinical specimens, such as urine, pus and sputum samples, from patients who came to the clinical biology laboratory of the Centre.

Muraz (Bobo-Dioulasso, Burkina Faso) for diagnostic testing. All strains used were grown on Chapman agar and incubated at 37˚C for 24 hours. Identification of *S. aureus* was performed using GRAM staining, mannitol fermentation, catalase and the Vitek 2® Compact automated system (Biomérieux, Marcy l'Etoile, France) with the GP67 card. All strains were stored at −80˚C in STGG medium for subsequent analyses. Identification and antibiotic susceptibility testing were performed using the Vitek 2 compact automated system with MRSA phenotype detection software in accordance with EUCAST 2022 recommendations [18]. All strains with a positive screening for cefoxitin were identified as MRSA. Three *S. aureus* reference strains, ATCC 29213 (susceptible strain), ATCC 43300 (MRSA strain) and ATCC 25923 (penicillin-resistant strain) were used as controls for the tests and were included in the phages host spectrum tests.

2.2. Collection and Treatment of Wastewater Samples

S. aureus specific lytic bacteriophages were isolated from five wastewater sites on the city of Bobo-Dioulasso (**Figure 1**), including the slaughterhouse of Nieneta, the end of drain station of Dogona, the water treatment plant of Dogona, the Souro Sanou University Hospital Centre (CHUSS) and the Houet backwater. These wastewaters were collected in 50 ml vial tubes and then sent to laboratory at Centre MURAZ (Centre MURAZ, National Institute of Health, Bobo-

Dioulasso, Burkina Faso) for further analysis. The wastewater was left to settle for one hour and then the supernatant was collected in a new 50 ml falcon tube and centrifuged at 3000 rpm at +4˚C for 5 minutes in Eppendorf 5804 R centrifuge (Eppendorf, Hamburg, Germany). The supernatant was filtered through 0.45 µm sterile filter. The filtrates were collected in new 50 ml vial tubes. The filtrate obtained was used directly for enrichment.

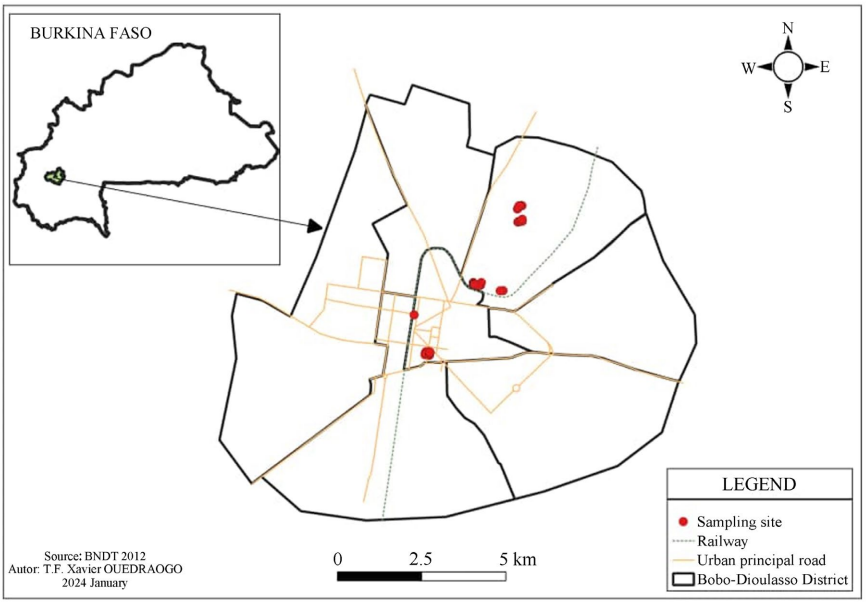


Figure 1. Map of wastewater collection sites in the city of Bobo Dioulasso (Source BNDT, 2012).

2.3. Bacteriophage Enrichment

Clinical (n = 6) and reference (n = 2) strains of *S. aureus* were previously inoculated on Chapman medium. A bacterial suspension equivalent to Mac Farland 0.5 was prepared using densiCHEK plus (BioMérieux, Marcy l'Etoile, France). 500 µl of each bacterial suspension was inoculated into a falcon tube containing 15 ml of Trypticase Soy Broth, 10 ml of wastewater filtrate and 63.75 µl of 2 mM CaCl2 and incubated at 37˚C for 48 hours. After incubation, the mixture was centrifuged at +4˚C at 3000 rpm for 20 minutes on a rotina 380 R Centrifuge (Hettich GmbH, Tuttlingen, Germany). The supernatant was filtered through 0.22 µm filter.

2.4. Phage Isolation and Purification

10 microliters of phage filtrate were deposited on the double layer of Luria-Bertani (LB) agar and incubated at 37˚C for 24 hours. The double layer of agar consisted of a soft agar (0.7% agar) previously inoculated with a bacterial suspension (host bacteria) and a solid agar (1.5% agar). After incubation, the lysis plaques were collected in a cryotube containing 1 ml of Phosphate bovine solution (PBS) and 10 µl of chloroform then centrifuged at 3000 rpm for 10 minutes to remove

bacterial debris. The supernatant is filtered using the 0.22 µm filters and collected in a new tube and stored at +4˚C [19].

Phage lysates obtained by the spot method were purified according to the protocol described by Lu *et al.* [20]. The phage lysates were then diluted in cascade in different dilutions of 2, ranging from 10^{-2} to 10^{-8}. Then 10 µl of the phage lysate from the last dilution, and 500 µl of bacterial suspension added to 10 µl 2 mM CaCl2 were homogenized in a hemolysis tube containing 4 ml of soft agar. The mixture was poured onto the solid LB agar solid and allowed to solidify at laboratory temperature. After 24 hours of incubation at 37˚C, the different lysis ranges obtained were distinguished macroscopically according to size (small and large) and appearance (clear and cloudy). The lysis ranges of the different phages were collected and then conditioned in PBS. The procedure was repeated three times until a uniform lysis range was obtained. Individual phages were stored in PBS at +4˚C [21] for further analysis.

2.5. Host Range of Phages

Determination of the host spectrum of purified phages was done using spot test [22] and the double layer method. Briefly, 500 µl of each bacterial suspension was mixed in a tube containing 4 ml of soft agar and then poured into the LB agar medium. After solidification, 5 µl of each purified phage lysate was pipetted and placed in contact with the bacteria, then the suspension was incubated at 37˚C for 18 - 24 hours and the presence or absence of lytic plaques was determined. The lytic activity of various bacteriophages isolated in the laboratory was tested on 14 MRSA and 02 *S. aureus* strains.

2.6. Extraction and Enzymatic Restriction of Phage Genomes

The double-layer LB agar containing the phage lysis plaques was covered with PBS buffer and stored at +4˚C for 24 hours. The PBS buffer was collected in a 15 ml falcon tube and filtered using 0.22 um filters. After filtration 10% polyethylene glycol was added to the phage buffer and incubated at +4˚C overnight. The mixture was centrifuged at 18,000 g at +4˚C for 15 minutes and the pellet was collected in a 1.5 ml eppendorf tube. Then, the pellet was suspended in 500 ul of buffer (100 mM Nacl, 10 mM MgSO4, 10 mM Tris HCL, distilled water), in which 1.5 ul DNase was added and incubated at 37˚C for 30 minutes. The rest of the extraction steps were carried out according to the Sambrook and Russell protocol [23]. The extracted DNA was assayed for purity using a nanodrop lite spectrophotometer (Thermo scientific, Waltham, Massachusetts, USA).

To discriminate between phages with the same host spectrum, the genetic fingerprints of these phages were determined by enzymatic restriction using BamHI and PstI according to the manufacturer's recommendations (Thermo Scientific, Waltham, Massachusetts, USA). The DNA fragments were then separated by agarose gel electrophoresis at 1% colored with ethidium bromide in Tris-acetate-EDTA buffer at 60 V for 3 hours.

3. Results

3.1. Drug Susceptibility Test of the Clinical Isolates and Reference Strains of *S. aureus*

We used in total n = 16 *S. aureus* strains (2 reference strains and 6 clinical strains in the isolation step plus 8 additional strains in the host spectrum step) in this study and Phenotypic DST was performed for 13 clinical isolates and the 2 reference strains (except the reference strain ATCC 25923).

The results of phenotypic DST show that the clinical strains and the reference strain ATCC 43300 used in this study were methicillin-resistant *S. aureus* with associated resistance to other families of antibiotics and the strain ATCC 29213 was a susceptible strain (Table 1).

3.2. Isolation of Lytic Phages of *S. aureus* by the Spot Method

Phages were isolated from the five sites in the city. Figure 2 shows lysis plates on TSA agar medium previously inoculated with the *S. aureus* strain. Figure 2 shows lysis plates on TSA agar medium previously inoculated with the *S. aureus* strain.

Table 2 describes the distribution of *S. aureus* specific phages across all five study sites in Bobo-Dioulasso. Phages were recovered from all sites. Phages from the abattoir lysed all 8 *S. aureus* strains used for the isolation.

Table 1. Antibiotic susceptibility test results for *S. aureus* strains.

Lab Id Bacteria	Type Sample	Antibiotic susceptibility test															
		FOX	PG	OX	GN	CIP	LV	MOX	E	CD	QP/DP	LIN	VA	TET	TG	NIT	SXT
29213	ATCC	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
43300	ATCC	R	R	R	R	I	I	S	R	R	S	S	R	S	S	S	S
SA_CM01	Pus	R	R	R	S	R	R	R	R	R	R	R	R	R	S	S	S
SA_CM02	Pus	R	R	R	S	I	I	R	R	R	R	R	R	R	S	S	S
SA_CM03	Sputum	R	R	R	S	I	I	R	R	R	R	R	R	R	S	S	S
SA_CM04	Pus	R	R	R	S	I	I	R	R	R	R	R	R	R	S	S	R
SA_CM05	Urine	R	R	R	R	I	I	R	R	R	R	R	R	R	S	R	R
SA_CM06	Urine	R	R	R	S	R	R	R	R	S	S	S	R	S	S	S	S
SA_CM07	Pus	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S
SA_CM08	Pus	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R
SA_CM09	Pus	R	R	R	S	I	I	R	R	R	R	R	R	R	S	S	S
SA_CM10	Urines	R	R	R	S	R	R	R	R	R	R	R	R	R	S	S	S
SA_CM11	Urines	R	R	R	S	R	R	R	R	R	R	R	R	R	S	S	S
SA_CM12	Urines	R	R	R	S	I	I	R	R	R	R	R	R	R	S	R	R
SA_CM14	Urines	R	R	R	R	I	I	R	R	R	R	R	R	R	S	R	R

R: resistant, S: susceptible, I: intermediate, SA_CM: *S. aureus*_Centre Muraz; Fox: cefoxitin, BP: Benzylpenicillin, OX: oxacillin; GN: gentamycin; CIP: ciprofloxacin; LV: Levofloxacin; MOX: Moxifloxacin; E: erythromycin; CD:clindamycin; QP/QP: quinupristin/dalfopristin; LIN: Linezolid, VA: Vancomycin; TG: Tigecyclin; NIT: nitrofurantoïn; SXT: Trimethoprim/sulfamethoxazol.

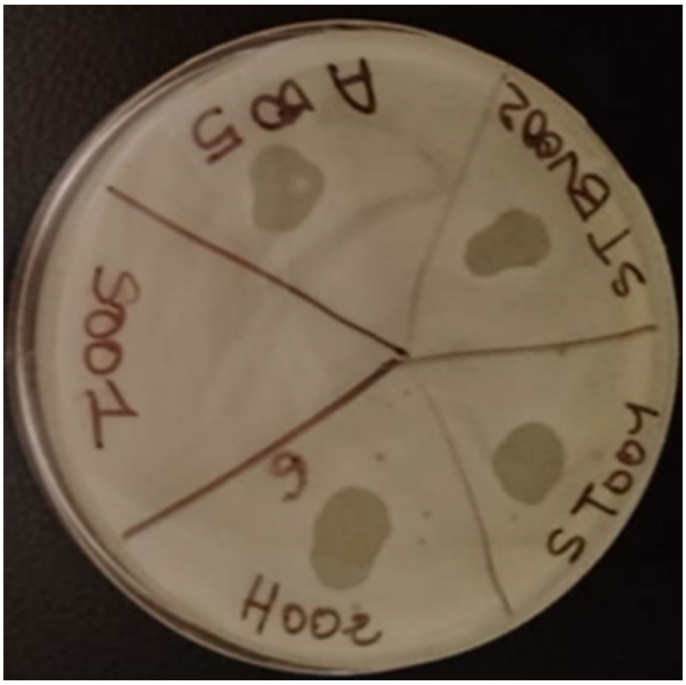


Figure 2. Morphology of *S aureus* lysis patches using the spot method.

Table 2. Distribution on the collection sites of *S. aureus* specific lytic phage.

Bacterial isolate	Collection site					Isolation frequencies
	CHUSS	WTPD	DESD	MH	SHN	
SA_CM01	-	-	+	+	+	3/5
SA_CM02	+	+	+	-	+	4/5
SA_CM03	+	+	+	-	+	4/5
SA_CM04	-	-	-	-	+	1/5
SA_CM06	+	+	+	-	+	4/5
SA_CM07	-	+	+	+	+	4/5
SA_CM08	+	-	-	-	+	2/5
SA_CM09	+	+	-	+	+	4/5

SA_CM: *S. aureus*_Centre MURAZ; CHUSS: Souro SANOU Hospital Center; WTPD: Water Treatment Plant of Dogona; DESD: Drainage End Station of Dogona; MH: Marigot Houet; SHN: Slaughterhouse of Nieneta.

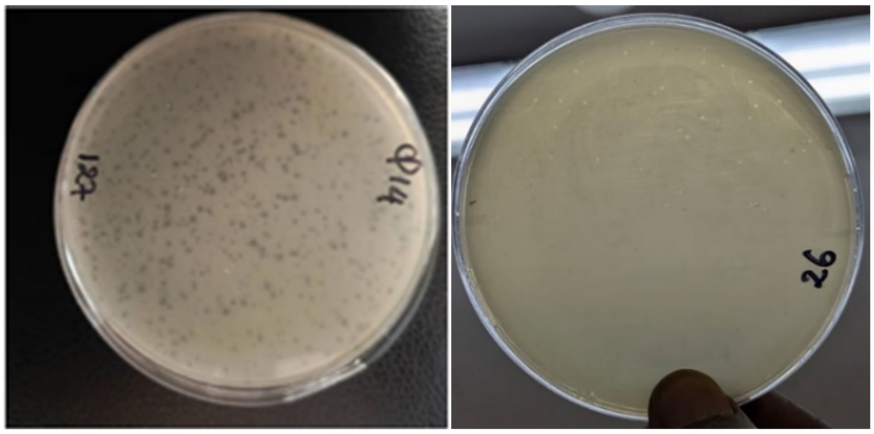
3.3. Number of Phages Isolated after Purification

Lysis Ranges of *S. aureus*

A total of 27 individual's lytic phages specific to *S. aureus* were isolated from Bobo-Dioulasso wastewater samples. These phages were divided into two groups according to their diameter size (Figure 3).

Diameter of bacteriophages plaque were appreciated by visual observation. Table 3 gives details of isolated phages by size. Small plaques of lysis were in the

majority.



(A) (B)

Figure 3. *S. aureus* specific phage after purification. A: larges plaques lysis; B: small plaques.

Table 3. Distribution of phages according to the size of the lysis plaque.

Bacteria	Number of different lysis phages (n = 27)	Diameter size	
		Small (n, %)	Large (n, %)
SA_CM01	3	03 (3/3)	00 (3/3)
SA_CM02	3	00 (3/3)	03 (3/3)
SA_CM03	4	03 (3/4)	01 (1/4)
SA_CM04	2	01(1/2)	01 (1/2)
SA_CM06	2	02 (2/2)	00 (2/2)
SA_CM07	2	02 (2/2)	00 (2/2)
SA_CM08	7	03 (3/7)	04 (4/7)
SA_CM09	4	03 (3/4)	01 (1/4)

3.4. Host Spectrum Results

This analysis highlighted the host range of the isolated phages. The host range of the 27 phages isolates was determined from 16 MRSA/SA strains using the spot and double layer method (Figure 4, Table 4). Except for the 8 host bacteria used for bacteriophage isolation, 8 other MRSA/SA strains were lysed by 7 isolated phages with the appearance of clear lysis plaques, visible to the naked eye on the agar.

Nine of 27 phage isolates are broad-spectrum (≥67%) and 7 phage isolates lysed 100% of bacteria (n = 16 *S. aureus* strains) used. But one phage isolate lysed five MRSA/SA; indicating a restricted host range of 31.25%. Then, the host range analysis allowed to obtain 15 distinct phages profiles. All the details of the results of the host spectrum of the isolated phages are summarised in the supplemental information's file (Table 5).

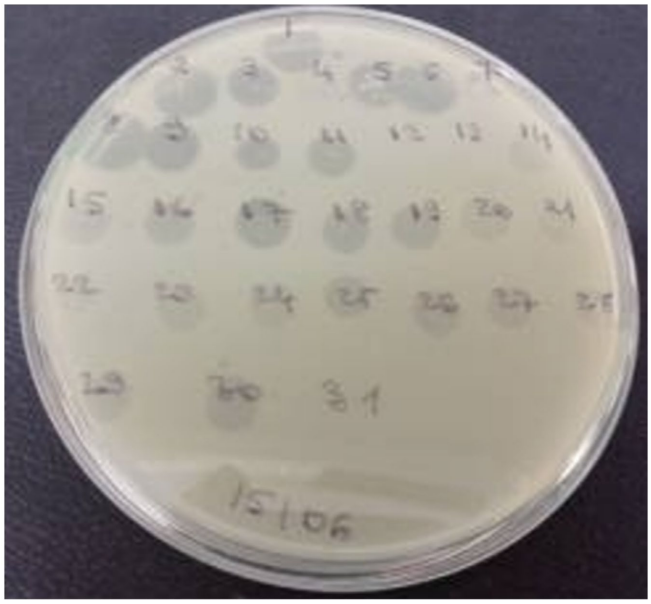


Figure 4. Host spectrum test by Spot Test method on strain SA_CM12.

Table 4. Correspondence between the phage identification number on the Petri dish and the codes assigned to the phage.

Corresponding table	phages Codes	Corresponding table	phages Codes
1	SA_BDS01	17	NA
2	SA_BDS0 2	18	SA_BDS18
3	SA_BDS03	19	SA_BDS19
4	SA_BDS04	20	SA_BDS20
5	SA_BDS05	21	SA_BDS21
6	SA_BDS06	22	SA_BDS22
7	SA_BDS07	23	SA_BDS23
8	SA_BDS08	24	SA_BDS24
9	SA_BDS09	25	SA_BDS25
10	SA_BDS10	26	SA_BDS26
11	SA_BDS11	27	SA_BDS27
12	NA	28	SA_BDS28
13	NA	29	SA_BDS29
14	SA_BDS14	30	SA_BDS30
15	SA_BDS15	31	NA
16	SA_BDS16		

Table 5. Results of host spectrum of isolates of *S. aureus* phages on the 16 bacterial strains.

	Bacteria Phages (N =27)	**Sau 01**	**Sau 02**	**Sau 03**	**Sau 04**	**Sau 05**	**Sau 06**	**Sau 07**	**Sau 08**	**Sau 09**	**Sau 10**	**Sau 11**	**Sau 12**	**Sau 13**	**Sau 14**	**Sau 15**	**Sau 16**
Pro 1	SA_BD_S01	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Continued

	SA_BD_S02	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	SA_BD_S05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pro 1	SA_BD_S06	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	SA_BD_S08	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	SA_BD_S09	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	SA_BD_S07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pro2	SA_BD_S03	+	−	+	+	+	+	+	**+**	**−**	+	+	+	+	+	+	+
Pro 3	SA_BD_S11	+	−	+	+	+	+	+	**−**	**+**	+	+	+	+	+	+	+
Pro4	SA_BD_S10	+	−	+	+	+	+	+	−	+	+	+	+	+	+	−	+
Pro5	SA_BD_S18	+	+	+	+	+	+	+	−	+	−	+	+	+	+	−	**−**
Pro6	SA_BD_S16	**−**	+	+	+	−	+	+	−	+	−	+	+	+	+	−	**+**
	SA_BD_S19	**−**	+	+	+	−	+	+	−	+	−	+	+	+	+	−	**+**
	SA_BD_S20	+	+	+	+	−	+	+	−	+	−	+	+	+	+	−	−
	SA_BD_S 25	+	+	+	+	−	+	+	−	+	−	+	+	+	+	−	−
Pro7	SA_BD_S26	+	+	+	+	−	+	+	−	+	−	+	+	+	+	−	−
	SA_BD_S027	+	+	+	+	−	+	+	−	+	−	+	+	+	+	−	−
	SA_BD_S28	+	+	+	+	−	+	+	−	+	−	+	+	+	+	−	−
	SA_BD_S30	+	+	+	+	−	+	+	−	+	−	+	+	+	+	−	−
Pro8	SA_BD_S29	+	−	+	+	−	+	+	−	+	−	+	+	+	+	−	−
Pro9	SA_BD_S21	+	−	+	+	−	+	+	−	−	−	+	+	+	+	−	−
Pro10	SA_BD_S15	−	−	**−**	+	−	+	**+**	−	+	−	+	+	+	**+**	−	−
Pro11	SA_BD_S22	+	−	**+**	+	−	+	**−**	−	+	−	+	+	+	**−**	−	−
Pro12	SA_BD_S24	−	−	**+**	+	−	+	**+**	−	+	−	+	+	+	**−**	−	−
Pro13	SA_BD_S14	−	−	**−**	+	−	+	+	−	+	−	+	+	+	−	−	−
Pro14	SA_BD_S23	−	−	**+**	+	−	+	−	−	+	−	+	+	+	−	−	−
Pro15	SA_BD_S04	−	−	**−**	+	+	+	+	−	+	−	−	−	−	−	−	−

Pro: Profil, **Sau**: *Staphylococcus aureus*; **SA_BD_S**: *Staphylococcus aureus*_ Bobo Dioulasso_Sewage, Sau01: SA_CM 01; Sau02: SA_CM 02; Sau03: SA_CM 03; Sau04: SA_CM04, Sau 05: SA_CM05; Sau 06: SA_CM06; Sau07: SA_CM07; Sau 08: ATCC 29231; Sau 09: SA_CM08; Sau 10: ATCC 25923; Sau11: SA_CM09; Sau 12: SA_CM10; Sau 13: SA_CM11; Sau 14: SA_CM12; Sau 15:ATCC 43300, Sau 16: SA_CM14.

3.5. Restriction Analysis of Phage Genome

The genetic fingerprints of the phages of each of the 15 profiles (supplemental Table 5) were compared with each other in order to highlight the difference at the molecular level of these phages. Two endonuclease enzymes including BamHI and Pst I were selected to determine the genetic fingerprints of phages with identical profiles. These enzymes were selected on the basis of in silico restriction enzyme tests from the whole genome of three specific *S. aureus* phages using the Malavida web tool https://serial-cloner.fr.malavida.com/windows/. The accession keys for

these phages are as follows MT787017.1, KY794642.1 And MN045228.1. These genomes were downloaded from the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The genetic fingerprints of the phages were determined by the mix of two enzymes. Analysis showed that phages from each profile had the same genetic fingerprint. **Figure 5** shows the genetic fingerprints of different phages from profile 1 and profile 7.

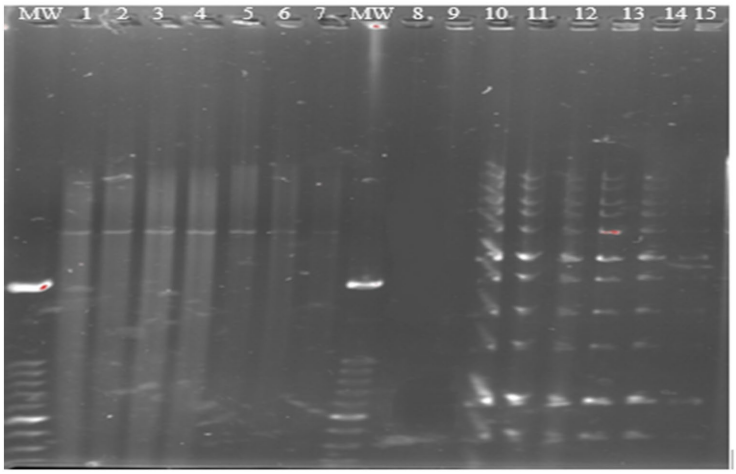


Figure 5. Agarose gel electrophoresis of enzymatic restriction fragments of phages genomes. MW: Molecular weight 1 kbp; Phages of profile 1 from 1 to 7. 1: SA_BD_S01; 2: SA_BD_S02; 3: SA_BD_S05; 4: SA_BD_S06; 5: SA_BD_S08; 6: SA_BD_S09; 7: SA_BD_S07.; 8: Negative Control; 9: Negative Control. Phages of profile 7 from 10 to 15. 10: SA_BD_S20; 11: SA_BD_S25; 12: SA_BD_S26; 13: SA_BD_S27; 14: SA_BD_S28; 15: SA_BD_S30.

4. Discussion

In this study, different cefoxitin-resistant *S. aureus* strains previously isolated in the Centre Muraz bacteriology laboratory were used as host bacteria for phage isolation in different environments in the city of Bobo-Dioulasso. Antibiotic susceptibility testing was performed to confirm susceptible or resistant status to antibiotic of all *S. aureus* strains used. These results confirmed that these clinical isolates were multiresistant strains. The multiresistance is a major public health concern, requiring reinforced surveillance and effective control measures to contain their proliferation [24]. The aim of this research is to isolate and characterize MRSA/SA-specific lytic phages from wastewater in the city of Bobo-Dioulasso. To our knowledge, this is the first study on the isolation of bacteriophages in Burkina Faso.

Phages were isolated from wastewater at all collection sites in the city of Bobo - Dioulasso. The results on isolation showed that the phages are widely distributed in Bobo-Dioulasso environments. This high distribution of phages in environment has been reported by the work of Díaz-Muñoz & Koskella in 2014 [25]. The abundance of phages in the wastewater sites could be explained by the fact that the wastewater contains sufficient host bacteria from the intersection of hospital

sewage, slaughterhouse, wastewater treatment plant, city end-of-drain plant and Houet backwater. Several studies have shown that Staphyphages were abundant in the hospital wastewater [26], fecal wastewater [27] and in wastewater treatment plants [15]. A similar study in China by Wang *et al.* in 2016 showed that *Staphylococcus* phages were abundant in fecal wastewater [27].

However, Matilla *et al.* (2015) in Finland showed that it was difficult to isolate *S. aureus* phages from environmental wastewater [28]. Furthermore, the study on phages biogeography states that phages are not abundant and uniform in all collection sites [29]. Phage abundance in the environment can be influenced by many factors such as site selection, temperature, amount of disinfectant substances used in wastewater, wastewater flow rate, exposure to sunlight or high radiation which can influence the amount of raw material [14] [15] [30].

Determination of the host range of phages allows to highlight the lytic capacity of staphyphages on a range of MRSA/SA strains. In this study 27 strains of MRSA/SA-specific phages were isolated. The host range of these phages was determined using spot tests that showed clear lysis plaques due to phage infection on host bacteria [29]. The results showed that all the strains used were lysed by 7 different MRSA/SA-specific phages. Thus, these 7 phages have a 100% broader host range. The broad host range phages obtained in our study may be of interest for phage therapy and healthcare applications. They can target multiple strains of bacteria of the same species, similar to the action of broad-spectrum antibiotics [30] [31] with the advantages to preserve the commensal flora [30]. This study on S aureus phages reinforces the literature on the use of phages against multi-resistant bacteria. Irrational use of antibiotics is a breeding ground for bacterial resistance to antibiotics, resulting in therapeutic failures in the care setting, often with side-effects and prolonged hospital stays [8]. The promise of phage therapy lies in its ability to lyse these multi-resistant bacteria, and its rapid, intense bactericidal action [32]. The selection pressure of phages is reduced, and their impact on ecosystems is limited by their specificity to a given bacterial species. The lysis effect of phage SA_BD_S04 killed 5 bacteria, indicating that its host range is narrow. This narrow-spectrum phage could be used to specifically target a given bacterial strain or in phages cocktails. In addition, phages were digested with endonucleases (BamH I, Pst I). The results of enzymatic digestion reveal that phages in profile 7 are identical and were cleaved by the mixture of two enzymes. Profile 1 phages, on the other hand, are also identical and resisted our two enzymes mixture digestion. Thus, based on the restriction fingerprints of phages genomes with the endonuclease enzymes (BamH I, Pst I), a total of 15 different phage isolates specific to MRSA/SA were isolated from the wastewater of the city of Bobo-Dioulasso. This study will pave the way for phage research in Burkina Faso by building the capacity of the phage bank for all aspects of basic and applied research.

5. Conclusion

This study is the first manuscript in Burkina Faso that describes the isolation and

preliminary of MRSA/SA lytic bacteriophages. These phages will be subject to whole-genome sequencing for their full characterization and functional genomics studies. Capacity building in all aspects of basic and applied phage research is needed in Burkina Faso. This will prepare the country with the capacity to produce its own phage cocktails for various health and other applications.

Authors Contribution

KG, M K G, R KY, K L W C E participated in the design and development of the study. K G and R K YAO carried out the analyses. All authors have read and approved the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] David, M.Z. and Daum, R.S. (2017) Treatment of *Staphylococcus aureus* Infections. In: *Current Topics in Microbiology and Immunology*, Springer, 325-383. https://doi.org/10.1007/82_2017_42

[2] Rocha, J.L.L., Kondo, W., Baptista, M.I.D.K., Cunha, C.A.D. and Martins, L.T.F. (2002) Uncommon Vancomycin: Induced Side Effects. *Brazilian Journal of Infectious Diseases*, **6**, 196-200. https://doi.org/10.1590/s1413-86702002000400007

[3] GBD 2019 Antimicrobial Resistance Collaborators (2022) Global Mortality Associated with 33 Bacterial Pathogens in 2019: A Systematic Analysis for the Global Burden of Disease Study 2019. *Lancet*, **400**, 2221-2248. https://doi.org/10.1016/S0140-6736(22)02185-7

[4] Price, R. (2016) O'neill Report on Antimicrobial Resistance: Funding for Antimicrobial Specialists Should Be Improved. *European Journal of Hospital Pharmacy*, **23**, 245-247. https://doi.org/10.1136/ejhpharm-2016-001013

[5] Ikuta, K.S., Swetschinski, L.R., Robles Aguilar, G., Sharara, F., Mestrovic, T., Gray, A.P., *et al.* (2022) Global Mortality Associated with 33 Bacterial Pathogens in 2019: A Systematic Analysis for the Global Burden of Disease Study 2019. *The Lancet*, **400**, 2221-2248. https://doi.org/10.1016/s0140-6736(22)02185-7

[6] Estrella, L.A., Quinones, J., Henry, M., Hannah, R.M., Pope, R.K., Hamilton, T., *et al.* (2016) Characterization of Novelstaphylococcus Aureuslytic Phage and Defining Their Combinatorial Virulence Using the Omnilog System. *Bacteriophage*, **6**, e1219440. https://doi.org/10.1080/21597081.2016.1219440

[7] Ouedraogo, A.S., Jean Pierre, H., Bañuls, A.L., Ouédraogo, R. and Godreuil, S. (2017) Emergence and Spread of Antibiotic Resistance in West Africa: Contributing Factors and Threat Assessment. *Médecine et Santé Tropicales*, **27**, 147-154. https://doi.org/10.1684/mst.2017.0678

[8] Paterson, D.L. and Bonomo, R.A. (2005) Extended-Spectrum β-Lactamases: A Clinical Update. *Clinical Microbiology Reviews*, **18**, 657-686. https://doi.org/10.1128/cmr.18.4.657-686.2005

[9] Ministry of Health and Public Hygiene (2023) Summary Report on Laboratory Surveillance of Antimicrobial Resistance in 2022, Ouagadougou, Burkina Faso.

[10] Ministry of Health and Public Hygiene (2024). Summary Report on Laboratory Surveillance of Antimicrobial Resistance in 2023, Ouagadougou, Burkina Faso.

[11] World Health Organization (2024) Proportion of Bloodstream Infection Due to Methicillin-Resistant *Staphylococcus aureus* (MRSA). https://data.who.int/indicators/i/5DD9606

[12] WHO (2021) Antimicrobial Resistance. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

[13] Nagel, T., Musila, L., Muthoni, M., Nikolich, M., Nakavuma, J.L. and Clokie, M.R. (2022) Phage Banks as Potential Tools to Rapidly and Cost-Effectively Manage Antimicrobial Resistance in the Developing World. *Current Opinion in Virology*, **53**, Article 101208. https://doi.org/10.1016/j.coviro.2022.101208

[14] Butler, M.S., Gigante, V., Sati, H., Paulin, S., Al-Sulaiman, L., Rex, J.H., *et al.* (2022) Analysis of the Clinical Pipeline of Treatments for Drug-Resistant Bacterial Infections: Despite Progress, More Action Is Needed. *Antimicrobial Agents and Chemotherapy*, **66**, e0199121. https://doi.org/10.1128/aac.01991-21

[15] Tan, C.S. (2020) Could Bacteriophages Isolated from the Sewage Be the Solution to Methicillin-Resistant *Staphylococcus aureus*? *Medical Journal of Malaysia*, **75**, 110-116.

[16] Abedon, S.T., García, P., Mullany, P. and Aminov, R. (2017) Editorial: Phage Therapy: Past, Present and Future. *Frontiers in Microbiology*, **8**, Article 981. https://doi.org/10.3389/fmicb.2017.00981

[17] Ross, A., Ward, S. and Hyman, P. (2016) More Is Better: Selecting for Broad Host Range Bacteriophages. *Frontiers in Microbiology*, **7**, Article 1352. https://doi.org/10.3389/fmicb.2016.01352

[18] Cattoir, V. (2022) French Microbiology Society/Europian Committee on Antimicrobial Susceptibility Testing V.1.0 Mai 2022.

[19] Khan, A. and Joshi, H. (2022) Simple Two-Step, High Yield Protocol for Isolation and Amplification of Bacteriophages against Methicillin-Resistant *Staphylococcus Aureus* (MRSA). *Current Protocols*, **2**, e395. https://doi.org/10.1002/cpz1.395

[20] Lu, Z., Breidt, F., Fleming, H.P., Altermann, E. and Klaenhammer, T.R. (2003) Isolation and Characterization of a Lactobacillus Plantarum Bacteriophage from a Cucumber Fermentation. *International Journal of Food Microbiology*, **84**, 225-235. https://doi.org/10.1016/s0168-1605(03)00111-9

[21] Lu, Z. and Breidt, F. (2015) Escherichia Coli O157:H7 Bacteriophage Isolated from an Industrial Cucumber Fermentation at High Acidity and Salinity. *Frontiers in*

Microbiology, **6**, Article 67. https://doi.org/10.3389/fmicb.2015.00067

[22] Kutter, E. (2009) Phage Host Range and Efficiency of Plating. In: *Methods in Molecular Biology*, Humana Press, 141-149. https://doi.org/10.1007/978-1-60327-164-6_14

[23] Sambrook, J. and Russell, D.W. (2001) Molecular Cloning: A Laboratory Manual. 3rd Edition, Cold Spring Harbor Laboratory Press.

[24] WHO (2024) WHO Bacterial Priority Pathogens List, 2024: Bacterial Pathogens of Public Health Importance to Guide Research, Development and Strategies to Prevent and Control Antimicrobial Resistance HO Bacterial Priority Pathogens List.

[25] Díaz-Muñoz, S.L. and Koskella, B. (2014) Bacteria–Phage Interactions in Natural Environments. In: *Advances in Applied Microbiology*, Elsevier, 135-183. https://doi.org/10.1016/b978-0-12-800259-9.00004-4

[26] Samir, S., El-Far, A., Okasha, H., Mahdy, R., Samir, F. and Nasr, S. (2022) Isolation and Characterization of Lytic Bacteriophages from Sewage at an Egyptian Tertiary Care Hospital against Methicillin-Resistant *Staphylococcus aureus* Clinical Isolates. *Saudi Journal of Biological Sciences*, **29**, 3097-3106. https://doi.org/10.1016/j.sjbs.2022.03.019

[27] Wang, S., Huang, X., Yang, J., Yang, D., Zhang, Y., Hou, Y., *et al.* (2023) Biocontrol of Methicillin-Resistant *Staphylococcus aureus* Using a Virulent Bacteriophage Derived from a Temperate One. *Microbiological Research*, **267**, Article 127258. https://doi.org/10.1016/j.micres.2022.127258

[28] Mattila, S., Ruotsalainen, P. and Jalasvuori, M. (2015) On-Demand Isolation of Bacteriophages against Drug-Resistant Bacteria for Personalized Phage Therapy. *Frontiers in Microbiology*, **6**, Article 1271. https://doi.org/10.3389/fmicb.2015.01271

[29] Thurber, R.V. (2009) Current Insights into Phage Biodiversity and Biogeography. *Current Opinion in Microbiology*, **12**, 582-587. https://doi.org/10.1016/j.mib.2009.08.008

[30] Ariom, T.O., Iroha, I.R., Moses, I.B., Iroha, C.S.U., Ude, I. and Kalu, A.C. (2019) Detection and Phenotypic Characterization of Methicillin-Resistant *Staphylococcus aureus* from Clinical and Community Samples in Abakaliki, Ebonyi State, Nigeria. *African Health Sciences*, **19**, Article 2026. https://doi.org/10.4314/ahs.v19i2.26

[31] Hyman, P. (2019) Phages for Phage Therapy: Isolation, Characterization, and Host Range Breadth. *Pharmaceuticals*, **12**, Article 35. https://doi.org/10.3390/ph12010035

[32] Ravat, F., Jault, P. and Gabard, J. (2015) Bacteriophages and Phage Therapy: Using Natural Viruses to Treat Bacterial Infections. *Annals of Burns and Fire Disasters*, **18**, 13-20.